Oligonucleotide Data Sheet

TECH NOTE: Spin tubes or plates briefly before opening.

[redacted]

Order Ref. 1121315

P.O.

[redacted]

Order Date 7/27/2015

Tube #	Seq Name	Seq 5' to 3'	OD	Vol. µl	Con. µM	nmol	µg	Len	MW	% GC Content	E260	Tm	Scale	Purity	Add x µl for 100 µM	Barcode ID
1	AFwd1	AATTCAAAAATCTAGACCAGATTGG AGAGACACTTGAG	25.4	dry		62.5	734.8	38	11751.8	36.8	406374	66.8	25 nmol	Salt-Free	625	1025190092
2	AFwd2	CAAAAATCTAGACCAGATTGGAGAG ACACTTGAG	25.5	dry		70.2	737.8	34	10517	41.2	363097	67.1	25 nmol	Salt-Free	702	1025190093
3	ARev	AACTGCAGAAAAAACTAGTGGAAGA TCCAGATGCTGAAGG	33.4	dry		76.6	952.9	40	12444.2	42.5	435737	69.8	25 nmol	Salt-Free	766	1025190094
4	BFwd	AAAAAGAATTCAAAAATCTAGACGCT TCGATCTGGGCTATTC	25.3	dry		56.4	754.3	42	12918.6	35.7	432602	67.6	25 nmol	Salt-Free	564	1025190095
5	BRev	AACTGCAGAAAAAACTAGTGTCTTG AAGTCCTCCGTCATC	26.5	dry		66.9	819	40	12246.1	42.5	396459	69.6	25 nmol	Salt-Free	669	1025190096
6	BFFwd	NNNNNGAATTCAAAAATCTAGAATG AAAGACAAGCCGCATATC	26	dry		56.6	749.9	43	13249.5	36	459712	68.1	25 nmol	Salt-Free	566	1025190097
7	FFwd1	AATTCNNNNNTCTAGACATTGAGCA CGGTCGATTTC	20.4	dry		58.9	649.5	38	11030	43.1	345569	68.7	25 nmol	Salt-Free	589	1025190098
8	FFwd2	CNNNNNTCTAGACATTGAGCACGGT CGATTTC	18	dry		59.7	584.3	39	9795.2	48.4	302232	69.1	25 nmol	Salt-Free	597	1025190099
9	FRev	AAAAACTGCAGAAAAAACTAGTGGC ATCTCGCTGATGATTTATG	31.5	dry		68.4	930.3	44	13596	36.4	460780	68.4	25 nmol	Salt-Free	684	1025190100
10	HFwd	AAAAAGAATTCAAANNTCTAGAGTG AATTCCAGTTGCTGTGTG	29.2	dry		65.3	869.5	43	13313.4	34.9	447261	67.6	25 nmol	Salt-Free	653	1025190101
11	HRev1	GNNNNNACTAGTGATACGGCTGACA GGAAACA	18.7	dry		56	555.4	32	9911.3	48.4	333732	69.1	25 nmol	Salt-Free	560	1025190102
12	HRev2	TGCAGAAAAAACTAGTGATACGGCT GACAGGAAACA	22.7	dry		57.7	644.7	36	11168.4	41.7	393211	68.1	25 nmol	Salt-Free	577	1025190103

Eurofins Genomics
12701 Plantside Drive
Louisville, KY 40299
Phone:(800)688-2248
Fax:(251)252-7794

Cond't don't have any match

Laborenzin Sp CP4

Order #: 8166271
Order Ref.: 1096634

FOB Shipping Point

Bill To:	Ship To:
Naval Research Laboratory Cbmse Baochuan Lin 4555 Overlook Ave. S.W. Washington 20375 DC US	Baochuan Lin Naval Research Laboratory 4555 Overlook Ave. S.W., Code 6920 Cbmse Washington 20375 DC US (202) 767-0289

A and H are an operon in Laborenzin

D and G are also an operon ⇒ theory A and H / D and G

Carrier	Ship Date	Order Date	Customer P.O. #	Customer No.
UPS Next Day Air	07-Jun-2016	06-Jun-2016	ECom 11200033245	3047706

		Item	Description			Date Shipped
1		44000022-F	Tube 1/18	10 nmol	Len. 42	06/07/2016
2	A	44000022-R	Tube 2/18	10 nmol	Len. 43	06/07/2016
3		703000038-F	Tube 3/18	10 nmol	Len. 42	06/07/2016
4	B	703000038-R	Tube 4/18	10 nmol	Len. 43	06/07/2016
5		69300040-F	Tube 5/18	10 nmol	Len. 42	06/07/2016
6	C	69300040-R	Tube 6/18	10 nmol	Len. 43	06/07/2016
7		657000001-F	Tube 7/18	10 nmol	Len. 42	06/07/2016
8	D	657000001-R	Tube 8/18	10 nmol	Len. 43	06/07/2016
9		SB-prep-3P-1	Tube 9/18	10 nmol	Len. 22	06/07/2016
10		SB-prep-2Ea	Tube 10/18	10 nmol	Len. 26	06/07/2016
11		678000023K-F	Tube 11/18	10 nmol	Len. 43	06/07/2016
12	E	678000023K-R	Tube 12/18	10 nmol	Len. 38	06/07/2016
13		703000038K-F	Tube 13/18	10 nmol	Len. 44	06/07/2016
14	F	703000038K-R	Tube 14/18	10 nmol	Len. 46	06/07/2016
15		561000004K-F	Tube 15/18	10 nmol	Len. 44	06/07/2016
16	G	561000004K-R	Tube 16/18	10 nmol	Len. 43	06/07/2016
17		44000022K-F	Tube 17/18	10 nmol	Len. 39	06/07/2016
18	H	44000022K-R	Tube 18/18	10 nmol	Len. 40	06/07/2016

A and B ⇒ Arc A

C and E ⇒ DNA binding protein from thermophile

D and F ⇒ PPA

Unclassified organism ⇒ sensor/his kinase

Arc B

PPA

Arc B

A 770 C 1623 E 1415 G 1408

B 778 D 611 F 1446 H 1489

03 Jun 2016
M. Pena & M.Winkle

GAATTC= EcoRI
CTGCAG=PstI

>4400002x-F-EcoRI
ACG AAT TCA TGA GCG ATC CGA GCC CGC ATA

>4400002x-R-PstI
AGC TGC AGC ATT CGG CGC TGT CGC TGA CGT

>7030000038-F-EcoRI
GGG AAT TCA TGA GCA TCA TGA TAC ACA AAG

>7030000038-R-PstI
GAC TGC AGT CAT GTT ATT TGC ACG TGC ACG

>6930004x-F-EcoRI
AGG AAT TCA TGC CCA ATC AAT GCC CGG CGT

>6930004x-R-PstI
GGC TGC AGT TAT AAG TCG ATA TCG AGT TTC

>6800000027-F-EcoRI
CAG AAT TCA TGC CCA ATC AAT GCC CGG CGT

>6800000027-R-PstI
GGC TGC AGT TAT AAG TCG ATA TCG AGT TTC

>6570000001-F-EcoRI
AGG AAT TCG TGA GCG GAA CGG AAA CGG CAG

>6570000001-R-PstI
AAC TGC AGT TAC TTC GGC GCG CGT TTC GCA

>5610000004-F-EcoRI
AAG AAT TCG TGA GCG GAA CGG AAA CGG CAG

>5610000004-R-PstI
AAC TGC AGT TAC TTC GGC GCG CGT TTC GCA

Project No. NJK 1c

Book No. _____ TITLE _____

From Page No. _____

- Used Qiagen PCR cleanup kit from 2010. Works Okay
- Nanodrop 2000 results

Results	GFP fragment	Prnd	PacrB
[DNA conc]	11.3 ng/ul	15.2 ng/ul	6.9 ng/ul
260/280	1.96	2.00	2.08

Thursday 12.4.14

- Restriction enzymes from NEB have not arrived and there is no KpnI to be in the building

- Will transform pBBR1MCS-2::gfp mut3 into WM3064

Transformation protocol

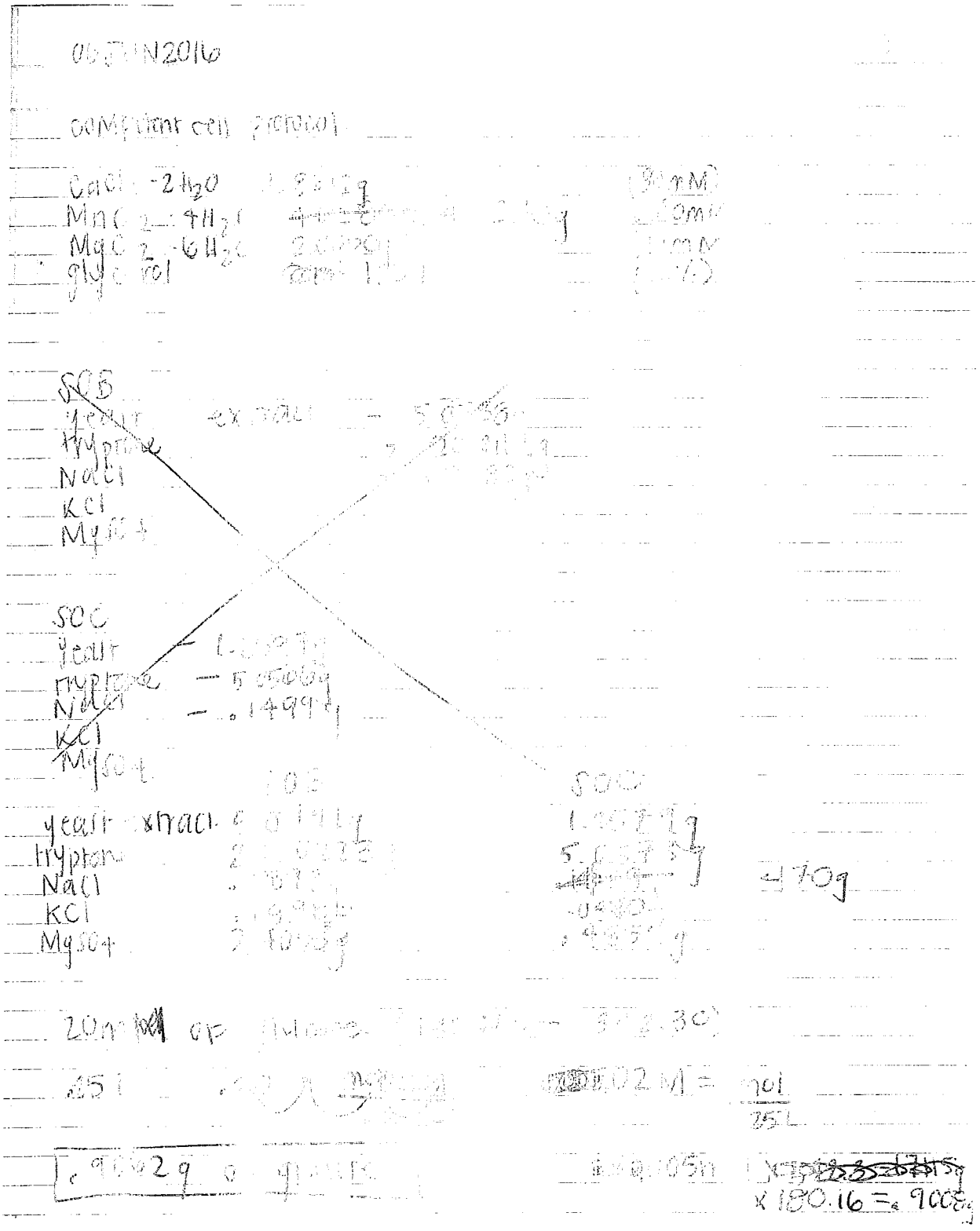
1. Remove competent cells from -80°C
2. Thaw on ice (20-30 min) 3 ul
3. Add 100pg-100ng (1-5ul) of DNA to (50)-100ul of competent cell
4. Put Incubate on ice for 30min
5. Heat Shock at 42°C for 3 min
6. Cool on ice for 5 min
7. ~~Add 750ul of LB~~ 50 ul of SOC
8. Incubate and shake for 1 hour at 37°C
9. Warm selective plates to 37°C
10. Spread 100ul DAP* onto 1 plate and spin down the rest.
11. Resuspend pellet in 200ul and plate
12. Incubate plates at 37°C O/N

* Only need DAP for WM3064 strains

Friday 12.5.14

- Transformation was successful.
  - ≈75 colonies on the LB+Kan DAP (100ul) Plate
  - ≈1000 colonies on the remaining plate.

Recorded by:	Date	Verified by:	Date



06JUN2016

COM; plant cell [illegible]

$CaCl_2 \cdot 2H_2O$	1.8892g		(30mM)
$MnCl_2 \cdot 4H_2O$	~~4.0050g~~	42.30g	(20mM)
$MgCl_2 \cdot 6H_2O$	20.0000g		(10mM)
glycerol	[illegible]		(10%)

SOB
yeast extract
tryptone
NaCl
KCl
$MgSO_4$

SOC
yeast
tryptone
NaCl
KCl
$MgSO_4$

SOB		SOC	
yeast extract	5.0171g	1.2579g	
tryptone	20.0223g	5.0573g	
NaCl	.4893g	~~.4384g~~	.1470g
KCl	.1867g	.0980g	
$MgSO_4$	2.4029g	.4855g	

20mM of glucose. [illegible] = 42.30)

.25 L [illegible] .02M = mol / 25L

.9062g of glucose

.005mol [illegible] x 180.16 = .900g

REMADE [illegible]

[redacted]

to make cells until they let DNA IN.

make competent cells out of E.coli — chemically competent cell
↳ put DNA inside. ↳ heat shock.

plasmid = small circular piece of DNA.
(~~good~~ easy to use)

- usually have antibiotic resistance gene.
(the only way to tell/tale)

4 antibiotics determined by iGEM

{ — — —

origin of replication

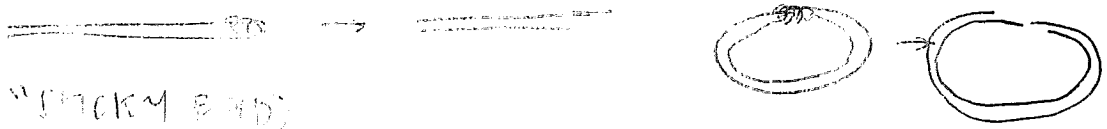
Multiple cloning site.
- short area w/ many different restriction sites.
(can't have restriction site IN genes)

take bacteria from culture & perform a DNA extraction.

~~- RNA~~ ~~- DNA~~

- RUN a PCR to make more of gene of interest.
  - primers bind on either side; polymerase COMES
    - Melt (95°C)
    - Anneal (primers bind)
    - extend (

- cut with restriction enzyme & cut plasmid with the same enzyme



"sticky ends"

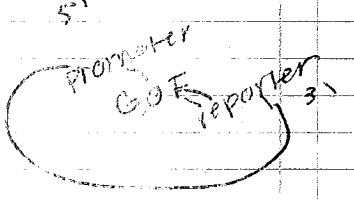
T4 DNA will connect & repair backbone so plasmid will be in tact = circle ← INA tube.

ligation is 90% efficient but since so much DNA = 10%

Plasmid get into competent cells

Placed on agar plate with antibiotic

(2) plasma will then add promoter ~~sequence~~ to make protein
- using restriction method



CONFIRMING protein
- main protein cells will survive on gel
- Look for band in your cells that is not present in any other one.

~~used~~ red Fluorescence (GFP/RFP)

(3) Add reporter (fluorescents) to early cell of protein to be expressed.
- Using restriction method

IN NORMAL LAB
- built in promoter in plasmid. (E.coli = standard promoter)

~~test~~ test competent cells w/ plasmid given in GEM kit.

absorbance of bacteria 9/7

8 am : .02
9 am : .026
10 am : .032
put into 37° incubator.
11 am : .101
12 pm : .163
12:15 : .304

Extraction of DNA 9/8
A -
B -

Primers ordered

44000022 - F & R ~~&~~ 4900022K-T & R - BIN 5
% Potential call @ ECORI (490).

ArcA

67800023K - F & R - BIN 8

70300035 - F & R & 703000356K - BIN 5

69800040 - F & R.
→ same primer used for 69000027 } NIF

69700001 - F & R. } PirA
56100004 - F & R.

plasmatr/ terminator gene search ON IGEM website

A & F th

- gfp reporter

Running PCR w/ primers ordered

Master Mixture

~~25uL x 8 = 200uL~~
~~+16uL~~

9C buffer	12.5uL buffer
9 template	1uL template
4.5 enzyme	.5uL enzyme
9C water	10uL water
	~~ground~~
	24 x 8.8

ladder: DNA marker 100-400bp

1st well: ladder
2-9 : A-H w/ 1uL of loading dye (xs concentration) & 5uL of sample.

96-25

9 ~~5~~uL of buffer.
~~9uL template.~~
~~4~~uL of enzyme.
~~3~~ uL of water.

} + 1uL of template
} + .5uL of each primer.

Oligonucleotide Data Sheet

TECH NOTE: Spin tubes or plates briefly before opening.

SpeI - ACTAGT 5
EcoRI - GAATTC 5
PstI - CTGCAG 2
XbaI - TCTAGA 5

[redacted]
Order Ref. 1096634
P.O.

[redacted]
Order Date 6/6/2016

	Type #	Seq Name	Seq 5' to 3'	OD	Vol. µl	Conc. µM	nmol	ug	Len	MW	% GC Content	ε260	Tm	Scale	Purif.	Add x µl for 100 µM	Order ID
A	1	44000022-F	GAATTCGCGGCCGCTTCTAGATGAG CGATCCGAGCCCGCATA	34.7	dry		87.2	1129.4	42	12900.4	59.5	397527	77.4	10 nmol	Salt-Free	872	1024994806
	2	44000022-R	TACTAGTAGCGGCCGCTGCAGCATT CGGCCGTGTCTCTCACTT	36.1	dry		91.4	1201.2	43	13135.6	58.1	385768	77.1	10 nmol	Salt-Free	914	1024994807
D	3	703000038-F	GAATTCGCGGCCGCTTCTAGATGAG CATCATGAAAGACAACC	36	dry		71.1	921.1	42	12956.6	50	422972	73.5	10 nmol	Salt-Free	711	1024994808
	4	703000038-R	TACTAGTAGCGGCCGCTGCAGTCAT GTTATTTCCACGTCCACG	26.3	dry		71.6	944.4	43	13145.6	53.5	394323	75.2	10 nmol	Salt-Free	716	1024994809
C	5	69300040-F	GAATTCGCGGCCGCTTCTAGATGCC CAATCAATGCCCGGCGT	30.1	dry		67.3	965.4	42	12956.6	59.5	387393	77.4	10 nmol	Salt-Free	673	1024994810
	6	69300040-R	TACTAGTAGCGGCCGCTGCAGTTAT AAGTCGATATCGAGTTTC	37.0	dry		90.6	1204.5	43	13276.7	46.5	418021	72.4	10 nmol	Salt-Free	906	1024994811
E	7	657000001-F	GAATTCGCGGCCGCTTCTAGATGCC CGCAAGCAAACCTCAC	28.1	dry		68.1	1001.1	42	13061.6	59.5	402192	77.4	10 nmol	Salt-Free	681	1024994812
	8	657000001-R	TACTAGTAGCGGCCGCTGCAGTTAC TTGGCGCGCGTTTCCCA	25.7	dry		66.9	869.7	43	13061.6	53.5	390292	75.1	10 nmol	Salt-Free	669	1024994813
	9	SB-prep-3P-1	GCCGCTGCAGTCCGGCAAAAA	15.1	dry		38.5	581.0	32	6733.4	65.1	230720	73.0	10 nmol	Salt-Free	385	1024994814
	10	SB-prep-3Fa	ATGAATTCCAGAAATCATCCTTAGC G	24	dry		92.5	742.8	25	7669.5	52.5	260192	74.4	10 nmol	Salt-Free	925	1024994567
XE	11	678000023K-F	GAATTCGCGGCCGCTTCTAGATGG CGCTGTTGAACAACTGCCA	27.1	dry		67.8	995.7	43	13224.6	55.1	405404	77.1	10 nmol	Salt-Free	678	1024994816
	12	678000023K-R	TACTAGTAGCGGCCGCTGCAGTCAT [illegible]	31.9	dry		81.7	1187.1	43	13135.6	55.8	350909	74.9	10 nmol	Salt-Free	817	1024994817
F	13	703000038K-F	GAATTCGCGGCCGCTTCTAGATGAT CAGCTGGTGGAAACGCAGT	30.7	dry		71.7	970.2	44	13612.9	54.5	428393	75.9	10 nmol	Salt-Free	717	1024994818
	14	703000038K-R	TACTAGTAGCGGCCGCTGCAGCTA CTGACAAGGTACGGTCAAAACG	31.9	dry		69.8	963.7	40	14193.2	54.3	456570	76.5	10 nmol	Salt-Free	698	1024994819

Oligonucleotide Data Sheet

TECH NOTE: Spin tubes or plates briefly before opening.

[redacted]

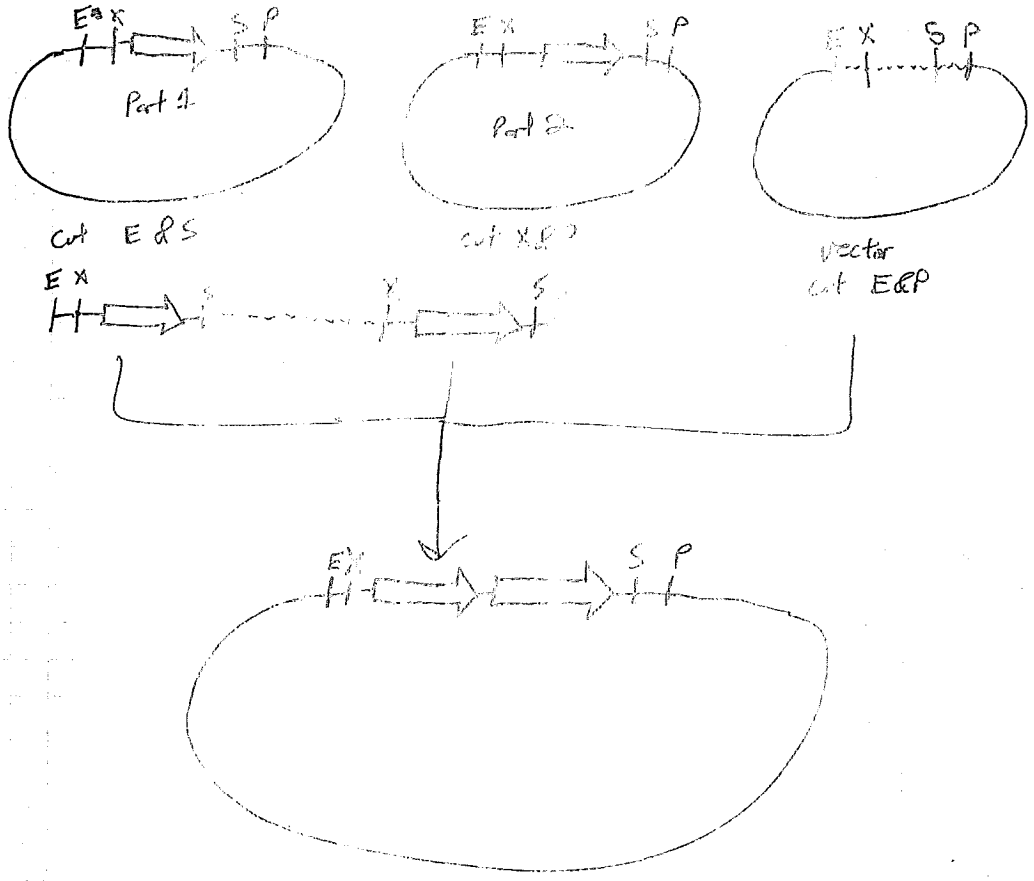
Order Ref. 1096534

P.O.

[redacted]

Order Date 6/6/2016

Tube #	Seq Name	Seq 5' to 3'	OD	Vol. µl	Dilution µl	nmol	µg	Tm	MW	% GC Content	ε260	T	Scale	Purif.	Add x µl for 100 nM	Barcode
15	561000004K-F	GAATTCGCGGCCGCTTCTAGATCG CGATCACACCATTACCAGC	29.5	dry		69.1	930.1	44	13481.2	50.0	410358	75.8	10 nmol	Salt-Free	691	10249748 20
16	561000004K-R	TACTAGTAGCGGCCGCTGCAGTTAT ACGTCCCGGCTGCTGGCT	32	dry		64	960		13 220.6	57.9	390292	75.1	10 nmol	Salt-Free	666	10249748 21
17	44000022K-F	GAATTCGCGGCCGCTTCTAGATCG GACACGTCCGACGC	15.8	dry		30.6	458.9	28	8 737.4	64.3	365407	74.1	10 nmol	Salt-Free	1633	10249748 22
18	44000022K-R	TACTAGTAGCGGCCGCTGCAGTTAT TTGCCCGCCCCGAGC	23.4	dry		73.6	809.1	40	10 975.5	65	358795	79	10 nmol	Sulfone	1392	10249748 23


E X
S P
Part 1
E X
S P
Part 2
E X
S P
cut E & S
cut X & P
vector
cut E&P
E X
X
S
E'
S P

Running PCR w/ primer added

Master Mixture

~~25 mL x 8 = 100 mL~~
~~+ 46 mL~~

1 C buffer
9 template
4.5 enzyme
9 C water

12.5 mL buffer
1 mL template
.5 mL enzyme
10 mL water
~~50 mL~~

24 x 8.8

ladder - DNA marker 100-1000 bp

1st well ladder
2-9 : A-H w/ 1 mL of loading dye (X5 concentration) &
5 mL of sample

96.25 97 mL of buffer.
~~Rax template~~
4 mL of enzyme.
~~5~~ 47 mL of water.

} + 1 mL of template
+ 5 mL of each primer.

ReMade SOC

Yeast extract	5.0045g
Tryptone	20.0013g
NaCl	
KCl	
MgSO4	2.4023g
glucose	3.9037g

Week 2

DAY 1:
- MADE LB + SOC → dark [illegible]
- MADE [illegible]

DAY 2:
- MADE competent cells → ab [illegible] (procedure adj
- MADE amp [illegible] agar

DAY 3:
- EXTRACTED [illegible] W/ DNA [illegible]
- Boiled competent cells (procedure [illegible]

DAY 4:
- Checked [illegible] NOT very competent (?)
- RAN PCR [illegible] DILUTIONS OF DNA
  W/ SAME [illegible] PRIMERS → NO dilution required.
- RAN PCR [illegible] (temp 70°C) 3hr

DAY 5.
- RAN Gel [illegible] 1st PCR → Bands were some w/ MM II band
- RAN PCR [illegible] (1st PCR used as template)
  [illegible] PCR temp @ 72°C → gel [illegible] good.
- SOC Media [illegible] (pg. 13)
- RAN PCR OF 2nd PCR (1st pr [illegible] as template) →
  Football color
- [illegible] Competent cells

Week 3:
DAY 6:
- RAN gel [illegible] PCR → Very faint bands
- Boiled Competent [illegible]
- Made [illegible] plates → 25 [illegible] LB agar + 1L H_2O NaCl → + chloramphenicol

Tetracycline, Ampicillin & Kanamycin

DAY 7.
- Checked new competent cells → Not so competent
- RAN gel of PCR products → DID NOT EVEN EVE. (LS)
- RAN PCR FOR restriction enzymes
- RAN another PCR for original PCR data again

5S [illegible] 3 = 15
5.5 x 10 = 55
5.5 x 1 = 3
3 Reverse
3 Forward

Sequence
- SF separate
- SR
- template

PCR DATA

PRACTICE PCR

PCR W/ 8 PRIMERS — TEMPLATE = DNA collected from biofilm
- 10.2 BMF
- 9 uL TEMPLATE
- 45 uL ENZYME
- 90 uL OF WATER

} 24 uL in each tube + .5 uL of forward primer + .5 uL of reverse primer

RUN @ 70°

PCR 1st attempt to clean up PCR results
- 97 uL of buffer
- 9 uL Enzyme
- 77 uL of water

} 23 uL in each tube + 1 uL of template from previous PCR (exclude 5) + .5 uL forward primer + .5 uL of reverse primer

RUN @ 72° — template = resultant corresponding PCR products

PCR 2nd attempt to clean up final PCR results
"same as above in 1st attempt"

RUN @

PCR
- 69 uL of buffer
- 3 uL Enzyme
- 55 uL of water
- 3 uL forward primer
- 3 uL reverse primer

} 24 uL in each tube (5) + 1 uL of template

TEMPLATE = from kit RUN @

.990g of Agrose + 40ml H_2O heated 4MIN → 2% gel

10mg of EtB NI 1ml of H_2O → 20uL added to agrose

Biocathode DNA
A - 1.02 x 109 ng/ml
B - 6.76 x 10⁹ ng/ml

PLASMIDS PCR:
4MIN EXTENSION
30 cycles 60°C

J - PSB1C3
K - PSB1A3
L - PSB1T3
M - PSB1K3
N - BBa_J04450 50pg/uL

J K L M N Ladder A — 11 labeled

0.0546g agrose → 1.5% gel
+ 20uL

loading dye
3ml glycerol
25mg orange G
7ml H_2O

TRANSFORMATIONS

Plate 4: 4G - Ribosome binding site
↳ chloramphenicol

2 - 3 ng of DNA in each well
↳ 200-300 pg per uL
↓
use 10uL of H_2O.
↓
TRANSFORM 1uL OF that.

1 qFP
3 promoters
1 ribosomes
↳ ON chloramphenicol plate

A - AMP
T - TETRA
K - KANAMYCIN
C - Chloramphenicol

Steps

Week 3

Day 4:

- Extracted DNA from pUC18 → using QIAprep Miniprep Kit
- Ran LS gel of plasmids & primer : DID NOT TURN out
- Ran 3rd PCR of primers
- Ran 2nd PCR of plasmids.
- Ran out 3rd PCR primer product on LS gel.
- Suspended RBS, Reporter & Promoter from IGEM wells & transformed into competent cells
  Made loading gel.
- Extracted DNA from A, C, F using QIA gel extraction kit.
- Transformed pUC18 in competent cells.

Setup & ran 4th primer PCR.

- plated transformations of reporters/promoters/RBS/pUC.

Day 5.

- Ran 4th primer PCR & 2nd plasmid PCR on gel.
- Extracted DNA from D, & all plasmids (in case more is needed later on)
- Digested PCR A C D F & plasmids
- Ligated digested A B C D F w/ digested tetracycline & kanamycin plasmid.
- Transformed ligations w/ competent cells & plated.
  - ↳ Modified protocol heat shock : 3 min
    incubate = 1 hr
    3 μL of ligation w/ 50 μL of cells.

Day [illegible]

- [illegible]
- [illegible]

[illegible] with new competent

[illegible] LB [illegible]

12 TUBES

4G.1 } chloramphenicol
4G.2 }

11P.1 } pCBIA1
11P.2 }

17B.1 } pCBIA2
17B.2 }

11G.1 } pSBIG3
11G.2 }

1=N.1 } pSBIG3
1=N.2 }

pUC18 → 1 MP

amount of digestion in ligation.

A = 40 ng/μL, 3 7 ng/μL

C = 4.2 ng/μL

3 D = 2.9 ng/μL

2 E = 13.2 ng/μL

A → 1.2 g 290 mg → 870 μL

C → 1.3 g 323 mg → 960 μL

F → 1.3 3 7 . mg → 1,120 μL

→ 270 L → 540 mg 1200

I → 42 mg 200 M → 440 mg 1200

→ 400 200

K → 4 7 mg 200

Pro[illegible] RE[illegible] gene GFP

- [illegible] gene & promoters → ~~the plasmid~~ ~~DNA~~ of template ~~for~~ PCR
- VARIED primers → Restriction Digest. (Digest & gel. No heat kill)

WEEK 4
DAY 2
- MINI[illegible] CHECKED PLATES ~~for~~ growth of HP (some possible GFP)
- [illegible] extraction of ApI, CpI, DpI, EpI
- Digest [illegible] ApI, CpI, DpI, EpI Prep.
- RAN[illegible] PCR product cut of Flagellin to check VARIED primer = No band
- RAN MUTAGENESIS PCR product cut on Flash gel = [illegible]
- Set up [illegible] RAN PCR OF MINIPIN w/ [illegible] primers

23 samples:
- [illegible] uL of BUFFER — 1 uL of template
- [illegible] uL of [illegible] — + .5 uL of forward
- [illegible] uL of AMP — + .5 uL of reverse

HP in AMP to maintain plasmid

- MAKE LIQUID CULTURE FOR GHP (X1)
- MAKE [illegible] LIQUID CULTURES FOR [illegible] transformed backbone (X5)
- BC[illegible] FOR [illegible] PRIMED primers

MIX: 35 uL template — 2 uL of enzyme — 24 + .5 of BUFFER
42
33

RAN PCR OF MINISPIN PCR PRODUCTS

DAY 3:
- RAN gel [illegible] PCR'S OF MINISPIN
  DIED [illegible] NUMBERS NO BANDS SHOWED
- Extracted DNA OF LIQUID CULTURES USING QIA MINI
- digested MINISPIN DNA
- RAN DIGESTED DNA ON large gel = NO BANDS
- digested MINISPIN DNA again.
- digested [illegible] promoter Reporter RBS ORF

DAY 4:
- RAN gel OF digested MINISPIN gene DNA:
  4 bands [illegible] A, C, F & P.
- RAN digest DNA ON gel gene DNA: band indicating plasmid w/ large inserts overall = good sign.
- digested [illegible] RBS & GOI
- ligated RBS-promoter → reporter & GOI
- [illegible] transformed ligations into competent cells
- plated [illegible] transformed cells

MM1	MM2
30uL BUFFER 2	50uL
3uL EcoRI	5uL
3uL PstI	5uL
H_4H_2O	190uL

C1 – 4.8
A1 – 0.6
T1 – 17.3
A1 – 7.9
A2 – 7.2
A3 – 46.
T3 – 25
T4 – 60.
A4 – 59.6
D5 – 3
C – 7
T5 – 6
G – 5
G – 2
T – 3
A3 – 0

C1 –
C2 –
C4 – 23.7 (3.61)
C5 –
D1 –
D2 –
D3 –
D4 – 1.6 (2.5) (4)
T1 – 4.5 (22.50) (4)
T2 – 20.5 (4.64) (4)
T3 – 8.1 (10.35)
T4 – 39.6 (9.53)

M1 –
M2 –
M3 –
M4 –
M5 –
M6 –

BLOCK 1 IGEM [illegible] transformation

INVENTORY

- Large bottle of [illegible] Buffer solution used in making competent cells
- iGEM competent cells
- iGEM linearized [illegible] control
- Orange box
  - [illegible] of [illegible] & Biocathode PMFC
  - [illegible] labeled A: [illegible] DNA extracted from Biocathode
  - [illegible] was done with it.
  - [illegible] labeled EtBr 10mg/ml used in agrose gel to see [illegible] under UV light.
  - [illegible] of DNA extracted from PMC18 bacteria — used to [illegible] competent cells
  - [illegible] of [illegible] from gel extraction & purification
  - [illegible] (I-M = corresponding to the gel extraction & purification of the [illegible] plasmids received by iGEM, in case we [illegible]
  - [illegible] J - pSB1AB, K - pSB1C3, L - pSB1K3.ml
  - M - 50µg [illegible] SB [illegible] Dissolved DNA in competent cell test kit.
- 6 iGEM plates — 6 wells opened & 5 well suspended / well [illegible] plate opened but not suspended.
- TUBE RACK
  - [illegible] of glycerol A, B, & F each injected with Kanamycin & Tetracycline respectively
  - 2 tubes [illegible] suspended in Chloramphenicol & Ampicillin.
  - [illegible] tubes [illegible] DNA extracted from liquid cultures.
  - [illegible] tubes for each generation of Tetracycline backebone labeled [illegible] (made on 6/22
  - [illegible] (where ... stands for A, B, C [illegible] culture made 6/21)
  - 2 [illegible] extracted for iGEM 1FN. W/ DpnI) [illegible] extracted from 4G & 1HP on 6/22
- 9 [illegible]
- 9 [illegible]
- [illegible]
- 28 [illegible]
  - [illegible] (created 6/22) & 1-5 1HP (created 6/22)
  - [illegible] 1-5 (created 6/22)

gel #:
A, A1-A5, C, [illegible] D5, [illegible] E, F, [illegible]

Digestion Master Mix

Primer Mixes
1. combos
→ 15N + 4
→ 11 Gq +

Beta-Gal-FP
1 A1-5 + 11 P
2 B1-5 + 11 P
3 [illegible]-5 + 11 P
4 [illegible]-5 + 11 P

3/N plasmid
A C 1-5 A1 C3 D
A1 A3 [illegible] Pf [illegible] 11 P
B1 11

16 plates of c
(Labeled w

5 sleeves of , , , w/ ciprofloxacin, 1 w/ Kanamycin,
1 w/ ampicillin

2. set [illegible]

Promoter → Control

pSpacer
Gal + GFP
↓ ↓
lacI cAMP.

w/ EcoO & PstI
tetracycline
chloramphenicol
(add DpN I)

X2 [illegible]
11 g + [illegible]

Digest w/ [illegible] Spe I Promoter & gene of interest.

3B = & GFP
Xba I & Pst

EcoR I & Spe
A
C
D
FS
IYN
11g

Xba I & Pst I
X 1 11P
X 2 4G

EcoR I & Pst I & DpN
2 tetracycline.
X4 of chloraphenicol.

10 total D[illegible]

EcoR I & SP
10mL 3
1mL of EcoR
1mL of Sp
3mL of W

Pst I
3 APTES
1mL of Pst I
3mL of Water

EcoR I + Pst I + DpN
10mL 3 APTES
1mL of EcoR I
1mL of PST I
1mL of DpN I
3mL of water